Inheritance and Frequency of a Color Polymorphism in *Danaus plexippus* (Lepidoptera: Danaidae) on Ohahu, Hawaii

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Abstract. A distinct color polymorphism has been observed in the Monarch butterfly *Danaus plexippus*, on Oahu, for at least the last 19 years. The rarer white morph, approximately 4% of the populations sampled in recent years, has grey-white rather than orange scales, but the pattern of its black scales is indistinguishable from that of the normal orange morph. Genetic crosses indicate that the white morph is homozygous for an autosomal recessive allele.

It is hypothesized that the white morph persists in Hawaii because it is not at a selective disadvantage to the orange morph in the presence of predators. Both morphs are captured by avian predators here in Hawaii, either because the milkweed on which the monarch larvae primarily feed (*Calotropis gigantea*) is low in or lacks certain cardiac glycosides, or because their principal vertebrate predators, *Pycnonotus* spp., are less susceptible to cardiac glycosides than other birds.

Introduction

Polymorphism, defined by Ford (1953), is the occurrence of two or more discontinuous forms of a species within one habitat in such proportions that the rarest form cannot be maintained solely by recurrent mutation. Examples of balanced and transient polymorphism are numerous in the Lepidoptera. Some of them involve Batesian mimicry of unpalatable species. Among the most famous examples are the transient polymorphism of *Panaxia dominula* L. (Fisher & Ford, 1947) and the transient and balanced polymorphism of *Biston betularia* L. (Kettlewell, 1958).

Color polymorphism has also been observed in the genus *Danaus*. Ford (1936) reported that Lamborn (1924) and Van Someren (1924, 1925) have studied the presence or absence of a subapical bar in the forewings of both sexes of *Danaus chrysippus* L. More recently Mitchell (1966) reported a "color" polymorphism in *Danaus plexippus* L. on Oahu, Hawaii.

Although the pattern of the black scales is indistinguishable in the two Hawaiian forms, the ground color of males and females of the white morph is cream-white to light-grey rather than orange. These mutants are depicted in Riotte and Uchida (1978). Urquhart (pers. comm.) noted "faded monarchs" during his studies of migration of *Danaus plexippus*, but did not consider them polymorphs. Faded areas on the wings of these monarchs can also be induced by microcauterizing the prismatic pigmented maculae of the pupa (Urquhart, 1972). Clarke and Rothschild (1980) worked with a mutant form of *Danaus plexippus* in which the adults possess areas of "whitish yellow" ground color. The ground colors of these two forms do not resemble that of the white monarchs found in Hawaii.

This paper reports on an investigation of the mode of inheritance of the white morph in Hawaiian *D. plexippus* and examines some of the possible selective factors that may have caused the high frequency and the increase in frequency of the white morph in Hawaii.

Methods

Crosses were carried out in the winter and spring of 1980, in the spring of 1983 and winter of 1983-1984, using adults that emerged from pupae collected from milkweeds (*Calotropis gigantea*) on the campus of the University of Hawaii. When their wings had dried following emergence, males and females were paired to effect the desired crosses. Pairs were kept in 40 cm x 26 cm x 30 cm wooden frame cages with top and sides constructed of either clear plastic or fine plastic screen that allowed sunlight to enter. The adults were maintained on a 10% sucrose solution. Eggs laid within the cages were removed on a fine paint brush, placed on fresh milkweed leaves, and put into petri dishes or vials until the larvae hatched. Larvae were allowed to feed on the leaves and grow to the third or fourth instar; they were then transferred to 30 cm x 15 cm x 10 cm plastic boxes. The boxes were examined and fresh leaves were fed to the larvae at least five days each week. Upon pupation, individuals were transferred from the larval containers into wooden frame cages. Newly emerged adults were removed from the cage and isolated to prevent mating. All rearing and mating cages were kept on a bench near a west facing window in broken sunlight in a well ventilated, non air-conditioned room.

A total of 23 crosses were initiated of which 16 produced a new generation of adults. Most larvae raised in the lab in the spring of 1980 and 1983, and a high proportion of larvae in the field were infected with a cytoplasmic polyhedrosis virus, and as a result only a small number of eggs were successfully raised to adults in the laboratory each generation. In the winter of 1983-84, only small numbers of eggs were obtained in most crosses, but larval mortality was very low.

Information concerning developmental times (egg, larval, and pupal

stages) was recorded for all F_1 and F_2 members in 1980.

Inter-year comparisons of the relative abundances of white and orange morphs were based on our own collections and data obtained from Mitchell (1966). Mitchell's data were based on "over 600 monarchs" reared from larvae and pupae he collected in Waianae and Hawaii Kai, Oahu, in February 1965. Our data were obtained by raising 522 pupae collected on the University of Hawaii campus (approx. 40 km E of Waianae and approx. 12 km W of Hawaii Kai) during the winter and spring of: 1972-1973, 1979-1980, 1980-1981, and 1982-1983; and in the winter of 1981. Monarchs become abundant in November-December and become inconspicuous around food plants by May or June each year on Oahu (Etchegary & Nishida, 1975).

Results

The white morph appears to follow a simple Mendelian mode of inheritance for an autosomal recessive allele (Table 1). All white-white crosses (crosses 1, 2, A) yielded only white offspring. The crosses between field orange and F_1 white individuals (crosses C, D, E, K) all produced orange offspring which were presumably heterozygotes. Crosses between white individuals and presumed heterozygotes (crosses F & G) yielded both white and orange offspring in ratios which were not significantly different from 1:1. Crosses H, I, and J only produced orange offspring.

The orange-white crosses of 1980 (crosses 3 & 4) gave rise to a mixture of both white and orange individuals. The orange individuals used in these crosses were probably heterozygotes. This would explain the near 1:1 ratio of white to normal coloration. Heterozygotes constituted about one-third of the field population in any year since 1972, assuming Hardy-Weinberg equilibrium (Table 2), so it is not improbable that the normal female and male in crosses 3 & 4 were heterozygotes. Very few individuals were produced by the one successful cross between two heterozygotes (cross L), but the occurrence of both white and orange offspring is consistent with the interpretation that the white allele is recessive.

Since the numbers produced by a cross are small, phenotypic ratios in filial generations are not known with any certainty. The heavy mortality of larvae may have altered the proportion of the white:orange ratio in 1980 if one morph was more susceptible to the virus than the other.

Times required for completion of the three main developmental stages were compared between the morphs in the 1980 crosses. Since no homozygous orange line was established, the duration of the egg stage of orange individuals was estimated by using the length of the egg stage for all eggs from white-orange crosses. No statistical comparison was performed on the length of the egg stage of the two morphs. Non-parametric statistical tests were made between the lengths of the larval developmental stages of the two morphs and no significant differences were found by a

Table 1. Results of crosses between laboratory reared *Danaus plexippus*. Parents were derived from pupae collected in the field or derived from laboratory crosses, except the white female of cross B which was collected in the field as a mated adult. Crosses 1-4 were performed in 1980, and crosses A-L were performed in 1983-1984.

Cross	Male	Female	Orange Male	Female	White Male	Female
1	White field	x White field	0	0	2	3
2	White F_1 of cross 1	x White F_1 of cross 1	0	0	3	2
3	White field	x Orange field	2	3	1	5
4	Orange field	x White F_1 of cross 1	1	3	7	3
A	White field	x White field	0	0	26	24
B	Unknown	x White field	19	15	19	14
C	White F_1 of cross B	x Orange field	7	6	0	0
D	Orange field	x White F_1 of cross B	7	3	0	0
E	White F_1 of cross B	x Orange field	6	8	0	0
F	Orange F_1 of cross B	x White F_1 of cross B	11	5	8	4
G	White F_1 of cross B	x Orange F_1 of cross B	6	8	8	5
H	Orange field	x Orange field	37	37		
I	Orange field	x Orange field	8	10		
J	Orange F_1 of cross H	x Orange F_1 of cross H	0	2		
K	White field	x Orange field	10	12		
L	Orange F_1 of cross K	x Orange F_1 of cross K	4	2	1	

Table 2.   Abundance of the white morph of *Danaus plexippus* in Oahu
          populations. Genotype frequencies are based on Hardy-
          Weinberg assumptions.

Date	Sample Size	Number White	Genotype NN[a]	Frequencies Nn[b]	nn[c]	Allele Frequency n
1965* (Feb)	600	3	0.863	0.132	0.005	0.071
1972 (Nov-Dec)	67	2				
1973 (Feb)	33	3	0.628	0.329	0.043	0.208
1973 (May)	16	0				
1979 (Dec)	43	2				
1980 (Jan)	42	2	0.681	0.288	0.030	0.175
1980 (Feb)	61	1				
1980 (Mar)	18	0				
1980 (Dec)	21	5				
1981 (Jan)	131	2	0.635	0.324	0.041	0.203
1981 (Feb)	77	3				
1981 (Mar)	13	0				
1981 Nov-Dec	177	6	0.672	0.294	0.034	0.180
1982 Dec	134	6				
1983 Feb-Mar	101	6	0.599	0.350	0.051	0.226

* Mitchell (1966)

a NN = orange, homozygous dominant

b Nn = orange, heterozygous

c nn = White, homozygous recessive

Mann-Whitney test. However, when the duration of the pupal stages were examined a significant difference was noted between the two forms at the 5% level. Since laboratory conditions were not carefully controlled with this type of comparison in mind, the significant difference noted between morphs in lengths of the pupal developmental periods cannot be accepted uncritically. There were no differences in the developmental periods between males and females within each of the two morphs.

Estimates of the frequency of white morphs on Oahu were obtained from Mitchell (1966) and from our own data and range between 0.5% and 5.1% (Table 2). Zimmerman (1958) estimated the earliest arrival of monarchs to Hawaii to be around 1845 when milkweeds were introduced; he made no mention of the existence of the white morph in Hawaii as of 1958.

A 2 x 4 $\times^2$ contingency analysis was performed to determine if the proportion of white morphs differed among the periods: 1965, 1972-73, 1979-80, and 1980-81. The test showed significant differences among the periods in the proportion of white individuals (p<0.01). When the 1965 data were compared against those for the combined periods of 1972-1973, 1979-1980, and 1980-1981 by a 2 x 2 contingency text, $\times^2$ was again significant (p<0.001). When a comparison was made among the years 1972-73, 1979-80, and 1980-81 by a 2 x 3 contingency table, no significant difference was found. Each of these tests is statistically independent of the other. From these results it appears that the ratio of white to orange has increased since 1965, but has not changed significantly during the last decade.

Discussion

The results of the crosses performed in this study indicate that the white trait is inherited as an autosomal recessive, similar to the observations of Clarke & Rothschild (1980) also in *Danaus plexippus*. However, Clarke & Rothschild suggest that the light morph they studied has a lower fecundity than the normal morph, whereas no pleiotropic effect has been documented in the Hawaiian white morph.

The analysis of phenotype frequencies indicates that the white morph may have increased between 1965 and the 1970's. There is some uncertainty about the increase because of the different sites sampled by Mitchell (1966) and ourselves. The proportion of white monarchs has probably increased since 1958, since it was not reported in Zimmerman's (1958) extensive monograph on Hawaiian insects, and because the earliest dates of collection of white specimens in 3 local museums are: 1965, 1967 and 1976. Since no change in frequency is apparent between 1972-1973 and 1982-1983, the genotype frequencies may have reached a balanced polymorphism. There is no evidence of any pleiotropy, differential mating, alteration of selection pressures, or heterozygote advantage which could explain the observed shift in gene and genotype frequencies. Matings between the two morphs have been observed in the field.

This white morph is evidently not restricted to Hawaii. A similar morph has appeared in Queensland, Australia (De Baar, 1982) and has been described for *Danaus plexippus* ssp. *erippus* from Argentina by Clarke & Rothschild (1980). Clark (1932) mentions that a specimen which may have been the white morph was captured outside the National Zoological Park in Washington, D. C. in 1896 by Capt. Robert Gill. Clark described

the specimen as, ". . .rather larger than usual and was entirely white on both sides."

The rarity of this white morph in North America may be due to predation on white individuals soon after emergence, because they are not recognized by vertebrate predators as unpalatable. Until recently the campus study sites and low altitude areas of the Hawaiian Islands have lacked avian predators which would eat larvae, pupae or adults of large lepidoptera such as monarchs. Since the spring of 1978, however, two bird species have appeared in numbers on the campus of the University of Hawaii and have been seen attacking larvae and orange and white adult monarchs in the area of campus milkweed stands. These birds, red-vented bulbuls (*Pycnonotus cafer*) and red-whiskered bulbuls (*Pycnonotus jocosus*), were first observed on Oahu in the mid-1960's (Berger, 1972). However, the bulbuls have actually never been seen ingesting the larval or adult monarchs. The wings are usually broken off the butterflies before the bird leaves the attack site carrying the adult body, possibly to nestlings. Both the white and normal orange monarchs are attacked in flight or at rest by the bulbuls. Thus, in Hawaii, it may be that both the mutant white individuals and the normal orange individuals have an equal probability of surviving to reproduce.

The observation that bulbuls attack both white and orange morphs suggest that both obtain insufficient cardiac glycosides from the local milkweed (*Calotropis gigantea*) to make them unpalatable to predators, or that *Pycnonotus* spp. are not susceptible to cardiac glycosides as in the case of grosbeaks (Fink & Brower, 1981). Preliminary analysis of the tissues of white and orange monarch butterflies raised on *Calotropis gigantea* in Hawaii indicate cardiac glycoside (cardenolides) concentrations are in the range of 140-180 ug/0.1 g of dry tissue (James Cohen, pers. comm.). This level in monarch populations of some geographic areas (Mexico, Massachusetts) is not quite sufficient to cause vomiting in in blue jays, the standard bio-assay animal (Fink & Brower, 1981).

The selective advantage conferred by the white allele which has allowed it to increase to a frequency of 20% is unknown. The maintenance of the white allele in the Hawaiian population is probably due to the fact that white individuals are not at a selective disadvantage here, whereas they may be at a selective disadvantage in North America, where they would be rare, unfamiliar to predators, and so killed despite having cardiac glycoside defenses.

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